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(54) Title: DIAGNOSIS OF KABUKI SYNDROME

(57) Abstract: A method for diagnosing an individual with a Kabuki syndrome related cytogenetic aberration is disclosed. The method includes the steps of providing an individual to be assessed for this aberration, obtaining a chromosome sample from the individual, and determining the presence or absence of a chromosome 8p22-8p23.1 duplication in the individual, wherein the presence of this duplication indicates that the individual possesses a Kabuki syndrome related cytogenetic aberration. For individuals exhibiting the cardinal clinical manifestations of Kabuki syndrome, determination of the presence of this duplication confirms a diagnosis of Kabuki syndrome. For others exhibiting related manifestations, determination of the presence of this duplication serves as a diagnosis of the Kabuki syndrome clinical spectrum. A related method, for testing for the presence of a heterozygous submicroscopic inversion at 8p23.1, outside of the duplicated region, is also disclosed. This cytogenetic aberration is detected in mothers of KS patients as well as in the patients themselves. All women, particularly all pregnant women, could be tested for the presence of this inversion for the purposes of genetic counseling.

TITLE OF INVENTION

DIAGNOSIS OF KABUKI SYNDROME

BACKGROUND OF THE INVENTION

Kabuki syndrome (KS, MIM147920) is a multiple congenital anomalies/mental retardation syndrome that is now well recognized worldwide with more than 350 cases reported in the literature^{1,2}. In 1981, Niikawa et al.³ and Kuroki et al.⁴ both initially described KS. The etiology of KS was undetermined, however, and clinicians had to rely upon cardinal clinical manifestations of this syndrome to make a diagnosis. Niikawa et al.⁵ delineated five cardinal manifestations of KS: 1) craniofacial anomalies characterized by long palpebral fissures with lateral eversion of the lower eyelids, arched eyebrows, depressed nasal tip, and prominent ears; 2) dermatoglyphic abnormalities including persistence of fetal fingertip pads and increased digital ulnar loops; 3) skeletal anomalies including brachydactyly and scoliosis; 4) postnatal growth deficiency; and 5) mild to moderate mental retardation.

Most cases of KS are sporadic with a nearly equal male-to-female ratio, but several familial cases have been reported with likely dominant transmission^{6,7}. Most patients with KS have normal karyotypes, although several different chromosome anomalies have been reported in individuals with features of KS^{1,2}, including some with ring X. However, the cardinal signs were not fulfilled in those cases, and they may represent a different clinical entity. Thus far, no autosomal, cytogenetic

aberrations have been determined in common among KS patients.

SUMMARY OF THE INVENTION

A chromosome 8p22-8p23.1 duplication ranging in size from about 0.68Mb to over 3Mb has been detected in unrelated KS patients representing different races. With the determination of a common etiological basis for this disorder, identification of a duplication in the indicated chromosomal region in an individual can be used in the diagnosis process.

Thus, the invention is directed to a method for diagnosing an individual with a Kabuki syndrome related cytogenetic aberration. The method includes the steps of providing an individual to be assessed for this aberration, obtaining a chromosome sample from the individual, and determining the presence or absence of a chromosome 8p22-8p23.1 duplication in the individual, wherein the presence of this duplication indicates that the individual possesses a Kabuki syndrome related cytogenetic aberration. For those individuals exhibiting the cardinal clinical manifestations of Kabuki syndrome, determination of the presence of this duplication confirms a diagnosis of Kabuki syndrome. For others exhibiting multiple congenital anomalies and/or mental retardation symptoms without exhibiting the cardinal clinical manifestations of Kabuki syndrome, determination of the presence of this duplication serves as a diagnosis of the Kabuki syndrome clinical spectrum. With both categories of individuals, family members should be tested for the presence or absence of the duplication.

Additionally, all tested KS patients and their mothers were determined to have a heterozygous submicroscopic inversion at 8p23.1, outside of the

duplicated region. Thus, in another aspect, the invention is directed to testing the same categories of individuals for the presence or absence of a heterozygous submicroscopic inversion at 8p23.1. Furthermore, all women, particularly all pregnant women, could be tested for the presence of this inversion for the purposes of genetic counseling.

BRIEF DESCRIPTION OF THE DRAWINGS

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims, taken in conjunction with the accompanying drawings, in which:

Fig. 1(a-f) shows CGH and BAC-FISH results on six KS cases using the diagnostic methods of the invention. The normal chromosome 8 is on the left side and the abnormal chromosome 8 is on the right side in each image in columns b - f.

a: CGH analysis showing enhanced copy number of chromosome segment 8p22-23.1.

b: RP11-235F10 (green) is not duplicated.

c: RP11-112G9 (red) is duplicated showing double signal on dup(8) chromosomes; RP11-23H1 (green) is not duplicated.

d: RP11-252K12 (green) is duplicated on dup(8) chromosomes.

e: RP11-31B7 (green) is duplicated on dup(8) chromosomes.

f: RP11-92C1 (green) is duplicated on dup(8) chromosomes;

Fig. 2(a-d) shows CGH and BAC-FISH comparison in KS case 1 and KS case 2 of Fig. 1 to their mothers (1m and 2m).

a: CGH showing no diminished or enhanced copy number of chromosome segment 8p22-23.1 in 1m and 2m, but showing enhanced copy number in 1 and 2.

b: One red signal (RP11-235F10) is located between split smaller green signals (RP11-122N11), one of which is inverted with (RP11-235F10) (arrow) in the interphases of both 1m and 2m as well as their affected children (1 and 2).

c: The same results as b on inv(8) (p23.1p23.1) metaphase chromosomes (right).

d: RP11-252K12 (green) is not duplicated on 1m and 2m inv(8) chromosomes (right), but is duplicated on both cases 1 and 2 dup(8) chromosomes (right) that also have an inversion;

Fig. 3(a-b) shows interphase and metaphase BAC-FISH, which revealed that the inv(8) (p23.1p23.1) and dup(8) (p22p23.1) events occurred on the same chromosome in cases 1 and 2.

a: Showing two split and inverted RP11-N11 (green) signals and two duplicated RP11-31B7 (red) signals are on the same chromosome in interphase (arrow) in both cases.

b: Same results as a confirmed on dup(8) metaphase chromosomes that also have the inversion;

Fig. 4 is an expansion of BAC-FISH probe analysis from 9.7 Mb to 12 Mb indicating a 0.68 Mb critical region and genes identified within that region; and

Fig. 5 is a diagnostic algorithm showing application of the results of the method of the invention.

DESCRIPTION OF THE INVENTION

The range of multiple anomalies and mental retardation in KS raises the possibility of a contiguous gene syndrome. Given the incidence of heart defects, cleft palate, and the occurrence of lower lip pits in KS, microdeletions involving chromosome 22q11 and chromosome 1q32-q41 have been investigated. Microdeletions involving these two regions have been excluded⁸⁻¹¹.

Comparative genomic hybridization (CGH) has proven useful in the past for demonstrating chromosome deletions and duplications related to other conditions¹²⁻¹⁵. Finding a microdeletion or microduplication among KS patients would be consistent with the mostly sporadic occurrence as well as the few reported cases of dominant inheritance of KS.

To test this hypothesis, CGH analysis was carried out on six patients who met the cardinal clinical diagnostic criteria for KS and in whom normal high-resolution chromosome analysis were documented. Four of the patients were Caucasian, one was African American, and one was Haitian¹⁶. These analyses revealed a chromosome 8p22-8p23.1 duplication in all of the patients studied, as shown in Fig. 1. Bacterial artificial chromosome - fluorescence *in situ* hybridization (BAC-FISH) was next employed to confirm and further define the duplicated region. At this stage, the duplicated region was delimited between BAC probes RP11-112G9 and RP11-92C1 and calculated to be approximately 3.5 Mb. CGH and high-resolution chromosome analysis from two sets of available parents all yielded normal results. However, the two mothers studied by BAC-FISH revealed an inversion of BAC probe RP11-122N11 at 8p23.1, as shown in Fig. 2. The remainder of the BAC-FISH studies from these parents showed normal results. Further BAC-FISH studies using

different fluorochromes revealed that the KS children of these two mothers inherited their mother's submicroscopic inversion at 8p23.1 and, furthermore, have a duplication involving the 8p22-8p23.1 region, as shown in Fig. 2. BAC-FISH studies of the remaining four KS patients using different fluorochromes revealed an identical submicroscopic inversion at 8p23.1. Furthermore, the inversion and the duplication of the 8p22-8p23.1 region were shown to be on the same chromosome (see Fig. 3). One out of 20 controls studied by BAC-FISH was found to have a heterozygous submicroscopic inversion at 8p22-8p23.1. This inversion is larger than that found in the KS patients and the two mothers studied but involves the common BAC-FISH probe, RP11-122N11. None of the controls showed duplication of the 8p22-8p23.1 region.

Kabuki syndrome families have continued to be recruited, and DNA and cell suspension samples have been obtained for 105 KS individuals and most of their parents. Referring now to Fig. 4, the Kabuki syndrome critical region has been refined by finding multiple patients with different sized duplications that overlap each other. A total of thirteen unrelated KS patients have been studied completely by CGH and additional BAC-FISH probes. Thirteen out of thirteen KS patients have duplication of four of the BAC probes. Nine out of thirteen KS patients have duplications of different sizes ranging from 0.85 Mb to 3.06 Mb. No deletions have been detected. Thirteen out of thirteen of the KS mothers and six out of six of the available KS fathers show no duplication for any of these BAC probes. All KS patients and their mothers continue to have a heterozygous submicroscopic inversion at 8p23.1. Based on the overlapping duplications in the unrelated KS patients, it

appears that the size of the minimum critical region has been narrowed to 0.68 Mb.

An inversion polymorphism in this general region between two 8p-Olfactory Receptor-gene clusters has been found in 26% of a population of European descent¹⁷. This finding implicates unequal crossing-over in meiosis in the maternal paracentric inversion carriers resulting in the duplication observed in their affected children. Although not observed in our cases, inverted duplications of 8p are well described and are often accompanied by a telomeric deletion (8p23.1-8pter)^{18,19}. Subtelomeric FISH, CGH, and BAC-FISH probes from 8p23.1-8pter, however, did not demonstrate a deletion in these KS patients.

Duplication of 8p23.2 has been reported by others as a normal variant²⁰. However, duplication of this region was not found in any of the KS patients. Controversy still exists as to the clinical significance of 8p23.1 duplications. These duplications have been documented in those with no or minimal pathology, as well as those with congenital heart defects, facial dysmorphism, and developmental delay²¹⁻²³. Duplications that include 8p22 have consistently resulted in phenotypic abnormalities that may vary with the extent of the duplication. These abnormalities have included mental retardation, facial dysmorphism, congenital heart defects, skeletal anomalies including scoliosis, and cleft lip and palate, all of which have been described in KS^{1,2,24,25}. Based on the findings reported here, it is proposed that KS may be a contiguous gene duplication syndrome of 8p22-8p23.1, whose molecular mechanism appears to be gene dosage, similar to Charcot-Marie-Tooth 1A²⁶.

In future studies, further refinement of the critical region 8p22-8p23.1 is to be expected. Results defining the molecular etiology of this duplication

(aiming to identify the precise molecular defect), results defining contiguous genes and their expression patterns and determination of genotype/phenotype correlations with potentially different sized duplications would be useful. Referring to Fig. 4, careful examination of the KS critical region using, e.g., FISH and DNA sequencing will be useful in order to test candidate genes and ESTs for their involvement in causing KS. As indicated in Fig. 4, several genes already have been identified in this region. These include B lymphoid tyrosine kinase (BLK), GATA binding protein 4 (GATA4), farnesyl-diphosphate farnesyl transferase 1 (FDFT1) and cathepsin B (CTSB). The location distances shown in Fig. 4 are based on the publicly available NCBI database as well as the Celera Corporation database. As there are several minor differences between the two, approximate distances are given. The GATA4 gene is a transcription factor essential for heart formation. Its expression has been demonstrated in heart, intestinal epithelium, primitive endoderm and gonads. GATA4 mutations have been found to cause human congenital septal heart defects and interact with TBX5 (T-box protein responsible for Holt-Oram syndrome)²⁹. GATA4 deficient mice fail to form a ventral heart tube and die of circulatory failure in early embryonic life. As half of KS individuals have cardiovascular anomalies, GATA4 is a strong candidate gene for genotype/phenotype correlations. Both CTSB and FDFT1 are expressed in the cochlea, making duplications of these genes possible candidates for the deafness that is found in 1/3 of KS patients. CTSB is also expressed in brain, pancreas, liver, kidney, skin, and several tumors and functions to cleave the amyloid beta-peptide. FDFT1 catalyzes the first step in the cholesterol

biosynthetic pathway. Mutations in this pathway have caused congenital abnormalities including dysmorphic craniofacies, skeletal abnormalities and mental retardation (e.g., Smith-Lemli-Opitz syndrome and Conradi-Hunermann-Happle syndrome)^{30,31}. BLK is expressed in B lymphocytes and immature T cells. It is thought to play an important role in signaling pathways and in thymopoiesis³². Involvement of this gene may lead to the immune dysfunction seen in some patients with Kabuki syndrome. Genomic FISH probes will be created from each of the identified candidate genes to detect whether any or all are involved in the previously detected duplication. Further refinement of this region will be made possible by identifying KS individuals with smaller duplications and eventually point mutations within one or more contiguous genes.

Genomic duplications are amenable to detection by various genetic methodologies. These methodologies, which are already being used for the detection of related disorders, include but are not limited to comparative genomic hybridization, fluorescence *in situ* hybridization, and polymerase chain reaction coupled with pulsed field gel electrophoresis for the detection of junctional fragments.

Clinical and Sample Collection - Original Analysis

All individuals involved in this study received careful examination by a clinical geneticist. Clinical information was furnished by each family who filled out the Kabuki syndrome clinical database survey²⁸ and provided several pictures of the affected individual. Blood was collected after IRB approval for high-resolution cytogenetic analysis, CGH and BAC-FISH analysis and submitted to the Center for Human Genetics

at Boston University School of Medicine. Twenty anonymized controls for BAC-FISH testing were selected. Ten samples (5 males; 5 females) were selected from those submitted to the laboratory for subtelomeric FISH analysis due to unexplained mental retardation. These individuals with mental retardation did not meet the cardinal diagnostic criteria for KS. Another 10 samples (5 males; 5 females) were selected from those submitted to the laboratory for routine chromosome analysis due to either multiple miscarriages or infertility. Genomic DNA was isolated from peripheral blood using the Pure Gene kit (Gentra Systems, Minneapolis, MN). Blood was also set up for FISH analysis by standard protocols.

Cytogenetic Analysis

Metaphase samples from patient, two sets of parents, and control peripheral blood lymphocytes were prepared according to standard procedures using methotrexate for synchronization of the cell cycle. Good quality metaphases of chromosomes were karyotyped by conventional G-banding at approximately 500-550 bands level.

CGH

Slides with normal lymphocyte metaphase chromosomes for CGH analysis were stored at -20°C before hybridization. CGH was performed essentially as described by Kirchhoff et al.¹³. Patient DNA and normal male or female reference DNA were labeled with FITC-12-dUTP and Texas Red-5-dUTP (DuPont, Boston, MA) respectively by using a nick translation labeling kit (Invitrogen, Carlsbad, CA). Genomic DNA was digested to fragment lengths of 0.3-2 kb. Labeled patient DNA (600 ng) and reference DNA (600 ng) together with excess unlabeled Human Cot-1 DNA (15 ug) (Invitrogen, Carlsbad, CA) were

precipitated and resuspended in hybridization buffer (50% formamide, 2xSSC, 10% dextran sulfate), then denatured in 70% formamide in 2xSSC at 73°C for 2 min. and hybridized to normal lymphocyte metaphase chromosomes denatured for 2 days at 37°C. Slides were washed in 0.4 x SSC with 0.3% NP-40 at 73°C for 2 min. and counterstained with DAPI II (4,6-diamidino-phenylindole in an anti-fade solution) (Vysis, Downers Grove, IL).

The CGH hybridization slides were analyzed using the CytoVision System Version 2.72 High Resolution CGH analysis software (Applied Imaging, Santa Clara, CA). 10-15 metaphases were captured using a Zeiss fluorescent microscope with an integrating CCD camera (Photometrics, Tucson, AZ). The green (patient DNA) to red (reference DNA) fluorescence ratio along the length of the chromosomes was calculated. The CGH profiles were compared to a dynamic standard reference interval based on an average of normal cases, as described by Kirchhoff et al^{12,13}. The intervals were scaled automatically to fit the test case. The mean ratio profile of each case with 99.5% confidence intervals was compared to the average ratio profile of the normal cases with similar confidence intervals. The aberrations found were those where the confidence intervals of the patient profile and normal averaged profile did not overlap.

FISH

10 BAC clones (RP11-11P7, RP11-140K14, RP11-122N11, RP11-235F10, RP11-112G9, RP11-252K12, RP11-31B7, RP11-92C1, RP11-23H1 and RP11-141K9) were selected from a human genomic library at the Children's Hospital Oakland Research Institute (Oakland, CA). BAC probes were isolated by standard procedure and labeled with FITC-12-dUTP or Texas Red-5-dUTP by standard nick translation.

The probe (60 ng) was precipitated with Human Cot-1 DNA (1 μ g) and resuspended in above hybridization buffer, then denatured and hybridized to prepared subject's metaphase slide overnight at 37°C. After post-wash, the chromosomes were counterstained with DAPI II.

USE

As shown in Fig. 5, a protocol has been developed, based on the experimental results reported herein, for using the methods of the invention, incorporating, e.g., CGH and BAC-FISH analysis, to detect the duplications and inversions as described herein for the diagnosis of KS and vulnerability to bearing a child having KS. The duplication has already been detected by BAC-FISH in both interphase and metaphase chromosome preparations. Detection can also be carried out by other FISH techniques, including, for example, the use of YACs, genomic and complimentary-DNA FISH probes, and fiber-FISH. Additional FISH probes will be used to attempt to detect even smaller duplications.

Any individual with several of the cardinal features of KS, which may include mental retardation, would be a candidate for testing by the methods reported herein. This testing will assist clinicians in making a precise diagnosis of KS as well as in providing accurate genetic counseling for families. In addition, as KS may be an underdiagnosed syndrome²⁷, it will potentially increase the number of patients recognized with this disorder, providing opportunities to better manage their care.

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While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

What is claimed is:

1. A method for diagnosing a Kabuki syndrome related cytogenetic aberration in an individual, said method comprising the steps of:

 providing an individual to be assessed for said aberration;

 obtaining a chromosome sample from said individual; and

 determining in said sample the presence or absence of a chromosome 8p22-8p23.1 duplication, wherein the presence of said duplication indicates that said individual possesses a Kabuki syndrome related cytogenetic aberration.

2. The method of claim 1, wherein said individual exhibits cardinal clinical manifestations of Kabuki syndrome and wherein determination of the presence of said duplication confirms a diagnosis of Kabuki syndrome.

3. The method of claim 1, wherein said individual is a family member of a patient exhibiting cardinal clinical manifestations of Kabuki syndrome.

4. The method of claim 1, wherein said individual exhibits multiple congenital anomalies and/or mental retardation symptoms without exhibiting cardinal clinical manifestations of Kabuki syndrome and wherein determination of the presence of said duplication serves as a diagnosis of the Kabuki syndrome clinical spectrum.

family member of a patient exhibiting multiple congenital anomalies and/or mental retardation symptoms without exhibiting cardinal clinical manifestations of Kabuki syndrome.

6. A method for diagnosing a Kabuki syndrome related cytogenetic aberration in an individual, said method comprising the steps of:

providing an individual to be assessed for said aberration;

obtaining a chromosome sample from said individual; and

determining in said sample the presence or absence of a heterozygous submicroscopic inversion at 8p23.1.

7. The method of claim 6, wherein said individual exhibits cardinal clinical manifestations of Kabuki syndrome.

8. The method of claim 6, wherein said individual is a family member of a patient exhibiting cardinal clinical manifestations of Kabuki syndrome.

9. The method of claim 6, wherein said individual exhibits multiple congenital anomalies and/or mental retardation symptoms without exhibiting cardinal clinical manifestations of Kabuki syndrome.

10. The method of claim 6, wherein said individual is a family member of a patient exhibiting multiple congenital anomalies and/or mental retardation symptoms without exhibiting cardinal clinical manifestations of Kabuki syndrome.

female.

12. The method of claim 11, wherein said individual is of childbearing age.

13. The method of claim 6, wherein said individual is a pregnant female.

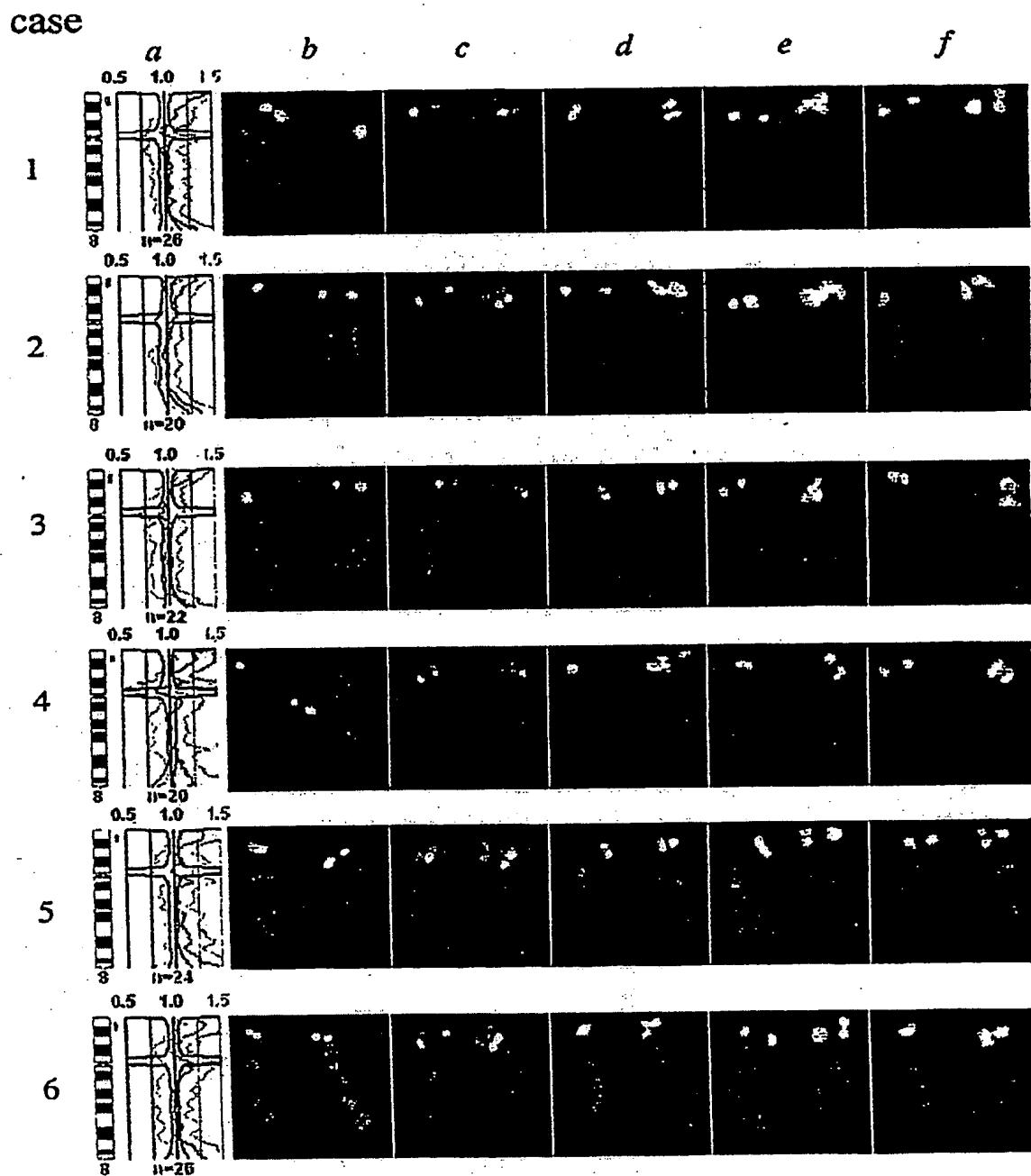
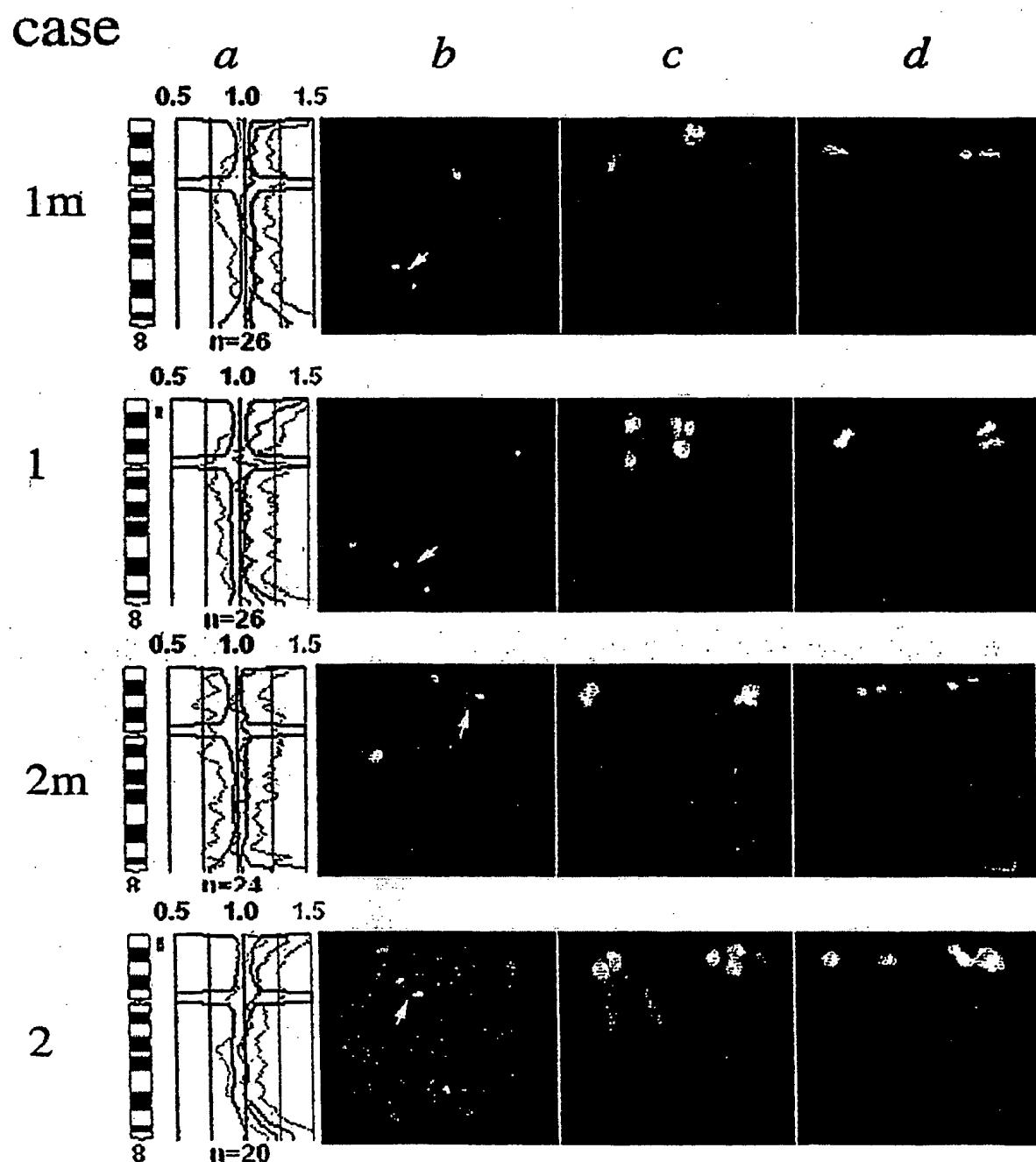


FIG. 1

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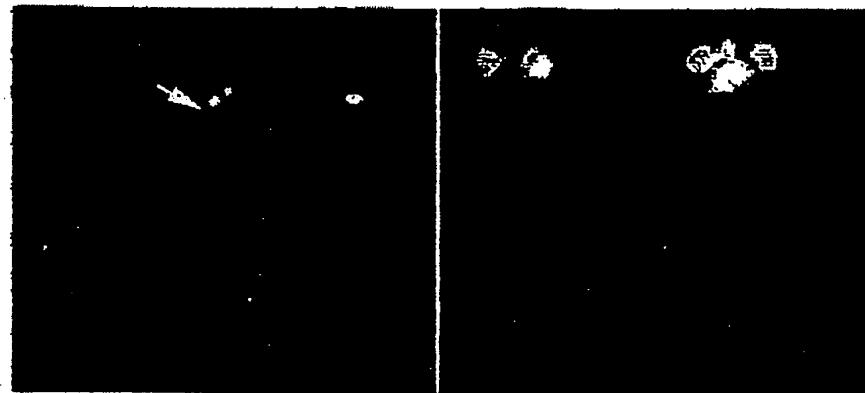
**FIG. 2**

case

a

b

1



2

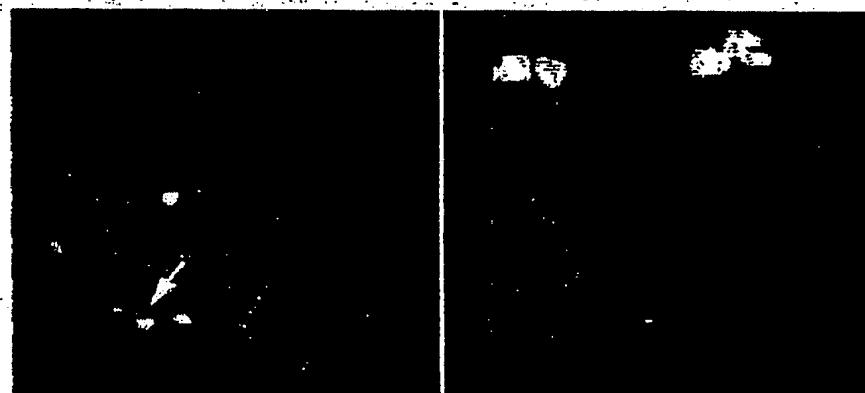
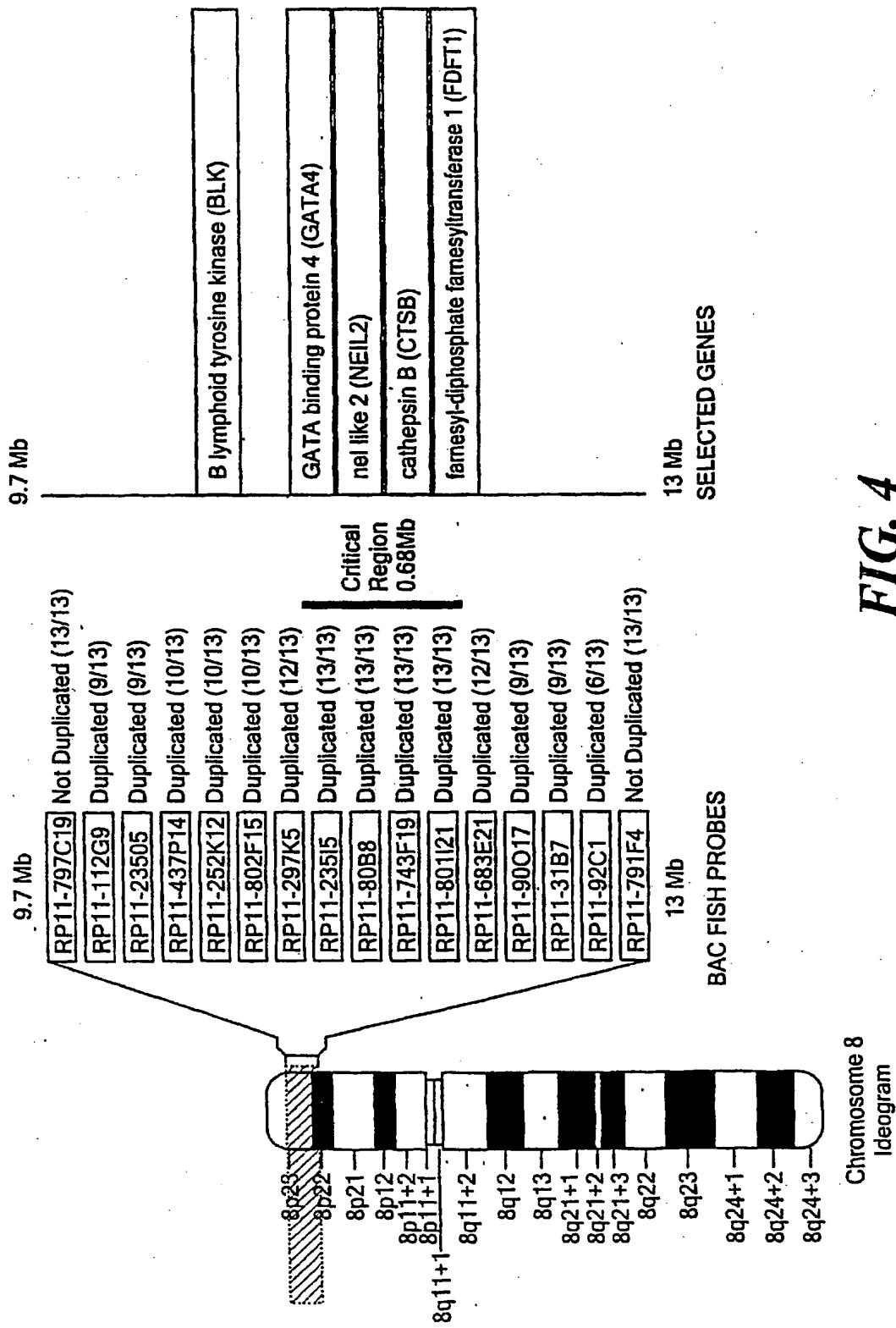


FIG. 3



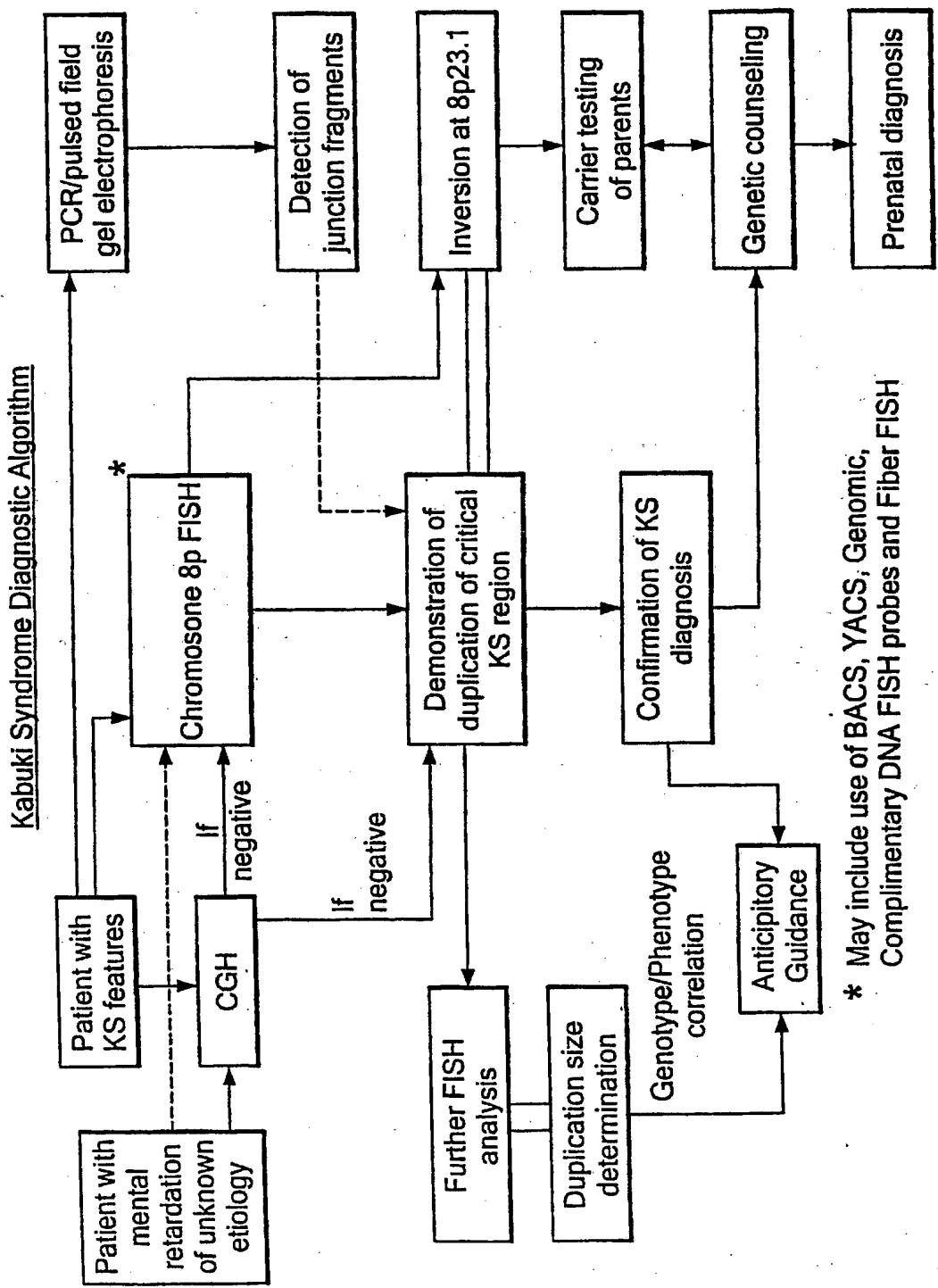


FIG. 5

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— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW. ARIPO patent (BW, GII, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations of inventorship (Rule 4.17(iv)) for US only

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) Title: DIAGNOSIS OF KABUKI SYNDROME

(57) Abstract: A method for diagnosing an individual with a Kabuki syndrome related cytogenetic aberration is disclosed. The method includes the steps of providing an individual to be assessed for this aberration, obtaining a chromosome sample from the individual, and determining the presence or absence of a chromosome 8p22-8p23.1 duplication in the individual, wherein the presence of this duplication indicates that the individual possesses a Kabuki syndrome related cytogenetic aberration. For individuals exhibiting the cardinal clinical manifestations of Kabuki syndrome, determination of the presence of this duplication confirms a diagnosis of Kabuki syndrome. For others exhibiting related manifestations, determination of the presence of this duplication serves as a diagnosis of the Kabuki syndrome clinical spectrum. A related method, for testing for the presence of a heterozygous submicroscopic inversion at 8p23.1, outside of the duplicated region, is also disclosed. This cytogenetic aberration is detected in mothers of KS patients as well as in the patients themselves. All women, particularly all pregnant women, could be tested for the presence of this inversion for the purposes of genetic counseling.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/19632

A. CLASSIFICATION OF SUBJECT MATTER

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US CL : 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	MILUNSKY, J.M. et al. Unmasking Kabuki Syndrome: chromosome 8p22-8p23.1 Duplication Revealed By Comparative Genomic Hybridization And BAC-FISH. Clinical Genetics. 2003, Vol 64, pages 509-516, see abstract.	1-11

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/19632

Continuation of B. FIELDS SEARCHED Item 3:
Meline, Caplus, East
search terms: Kabuki syndrome, chromosome, duplication, deletion, addition